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THE FUNGAL FLORA OF THE SUBMARINE ENVIRONMENT DURING PROLONGED SUBMERGENCE

by

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Bureau of Medicine and Surgery, Navy Department
Research Work Unit MF022.03.03-9025.31

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SUBMARINE MEDICAL RESEARCH LABORATORY
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SUMMARY PAGE

THE PROBLEM

To determine the fungal ecology of the submarine environment and to discuss its possible significance on the health of submarine crews.

FINDINGS

A brief literature review is presented in which it is determined that fungi, either as allergens or direct pathogens, do not contribute significantly to submarine crew morbidity. By means of cultures the primary components of the fungal ecology and the levels of fungal contamination throughout an FBM patrol are determined.

APPLICATION

This paper provides data concerning the fungal flora of the submarine environment for those interested in submarine microbial ecology.

ADMINISTRATIVE INFORMATION

This paper is submitted in partial completion of the requirements for designation "Qualified in Submarine Medicine." It has been selected for reproduction in order to make it available as study material in the School of Submarine Medicine, and as reference material in the Technical Library of the Submarine Medical Center. It has been designated as SubMedCen, SubMesResLab Report No. 535, under date of 17 June 1968 (Report No. 31 on BuMed Research Work Unit MF022.03.03-9025).

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ABSTRACT

Brief reviews are presented concerning present knowledge of the microscopic flora of the submarine environment as well as the role of fungal and allergic disease among FBM submarine crews. Data are presented which indicate that the numbers of air-borne fungi are markedly reduced during the course of a submerged patrol, and that this reduction is probably due to the removal of fungi and spores from the air by the air revitalization equipment. It is concluded that fungi probably do not play a significant role in FBM crew morbidity.

TABLE OF CONTENTS

TABLE OF ILLUSTRATIONS

THE FUNGAL FLORA OF THE SUBMARINE ENVIRONMENT DURING PROLONGED SUBMERGENCE

INTRODUCTION

The vast majority of noxious and obnoxious components of the submarine atmosphere and environment have been well enumerated. A large number of chemical constituents of the submarine atmosphere have been identified, both those present in vapor form and those present as particles in aerosols. The effects of most of these chemicals on man is known and limits for their concentration have been set to protect the health and well being of the crew.[1]

An equally important component of the submarine environment has been less well studied. This is the plant and animal ecology. The effects of microorganisms upon the health of the crew can be more significant than the effects of chemical contaminants.

Boyden has done a study of the bacterial flora of the FBM* submarine. He did counts of Streptococci, Micrococci, and Coliforms during an FBM patrol. He found the Micrococci to be the most prevalent air-borne organisms and Streptococci to be most prevalent in throat cultures. He noted that the highest concentration of air-borne bacteria was in the Operations Compartment and that the number of organisms in the air decreased after submerging for patrol. This was attributed to the removal of the organisms by the air revitalization equipment.[2]

Other important components of the submarine environmental flora are the fungi. These ubiquitous organisms are almost exclusively saprophytic with the exception of a small number of parasitic species. They can digest almost any organic compound. They require a high relative humidity, but under drought conditions produce spores which are an important component of dust.[3]

Fungi may be directly pathogenic to man, although very few species are in this category. Some of the normally saprophytic fungi may also become opportunistic parasites upon man, when the host is already debilitated. They may also appear as "overgrowth" organisms following steroid or antibiotic treatment. The more important fungal pathogens among the submarine population are those responsible for the superficial mycoses, the dermatophytes. Tinea pedis, or "Athlete's Foot" is the most prevalent of all dermatophytoses both in the general population and in the submarine population.[4, 5, 6] Hinshaw has studied the incidence of fungal disease on FBM submarine patrols and has found an incidence of 29.45 per 10,000 superficial mycoses, 2.16 per 10,000 deep mycoses, and 2.16 per thousand intermediate types. He found no difference in incidence between the on-crew and off-crew period or the refit and patrol period. He makes note of the chronicity and nuisance value of the superficial mycoses.[7]

Of equal importance in terms of morbidity due to fungi is their role in allergic disease. Fungi or their spores have been implicated as causative agents in both the more common allergic diseases such as hay fever and asthma, as well as the unusual ones such as Farmer's lung. Many of the common "harmless' or "beneficial" fungi are in this category, including Penicillium, Aspergillus, and Rhizopus.[6, 8] Kelley has studied the role of allergic disease on FBM submarines and noted that its incidence is quite low, even among "allergy prone" individuals. He did fungal cultures at two periods during an FBM patrol. He noted a decrease in air-borne fungi from 58 colonies on six plates in crew's berthing and 64 on six plates in the fan room at the time of submerging to five colonies on six plates in each area at mid-patrol. On Kelley's first set of plates a great variety of fungi were present, whereas on the second set Penicillium was the predominant organism.[9]

Because of the lack of information available on the submarine flora and the possible significance of fungi as a factor in submarine crew morbidity, it was felt that a study of

* Fleet Ballistic Missile (Polaris equipped)

the fungal flora of an FBM submarine during prolonged submergence would be a valuable contribution to the field of submarine medicine. The following study was, therefore, undertaken as partial completion of the requirements for designation as "Qualified in Submarine Medicine."

MATERIAL AND METHODS

All cultures were grown on Sabouraud Dextrose Agar (Bacto) which favors growth of fungi over bacteria by its acid reaction (pH 5.6).[10] Cultures were grown at room temperature (70° F.) a fact which further enhanced the selection of fungi in the cultures. Most fungi, with the exception of a few pathogenic types, grow well at room temperature; whereas most bacteria are inhibited by temperatures in this range. Media were prepared shortly prior to use by utilizing facilities of the USS SIMON LAKE during refit, and the facilities of the ship's Sick Bay while on patrol.

Two types of cultures were taken: 9 cm Petri dishes which were exposed to the atmosphere for a period of one hour, and 2-ounce medicine bottle slants which were streaked with swabs taken from areas suspected of harboring fungi. Six cultures of each type were taken, at approximately two-week intervals, from areas throughout the ship concentrating especially on the living spaces and the scrubber room. One set of cultures was taken during refit, another after surfacing from patrol, and the remaining four sets while submerged on patrol. One culture was taken on deck and one from a swab of Holy Loch water. A total of 36 plates and 30 swabs were taken.

All cultures were read after one week which seemed to provide the optimum in colony production without contiguous growth, although in some instances contiguous growth did occur in this time. When possible, colony counts were done. When contiguous growth had occurred, cultures were graded as light, moderate, or heavy growth.

The fungi cultured were classified according to gross colonial characteristics as well as microscopic characteristics of hyphae and spores. The great majority of colonies were Ascomycetes which were differentiated into three types: Penicillium, Aspergillus, and Yeasts. Ascomycetes are characterized by hyphae with cell walls, conidiospores at the ends of hyphae and asci containing eight ascospores. Yeasts are normally unicellular and reproduce by budding but do occasionally reproduce by means of ascospores. The only other fungus cultured was a Phycomycete, Rhizopus (black bread mold). This group is characterized by lack of cell walls in hypae and stalked sporangia. Figure 1 illustrates the microscopic morphology of the fungi cultured.[3, 8]

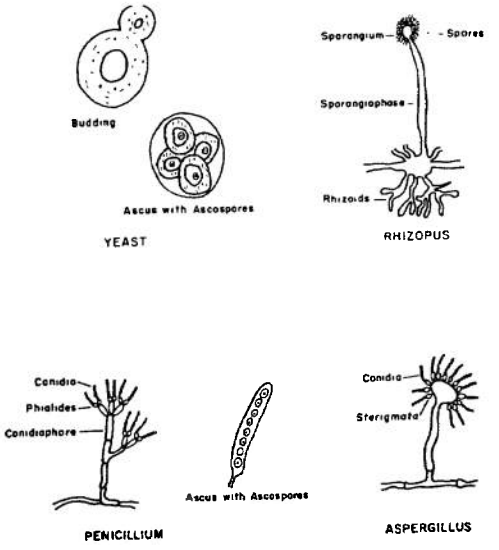

Figure 1. — Typical Fungi

RESULTS

Part I: Analysis of Air-borne Fungi

Analysis of air-borne fungi was carried out by the settling plate technique which provides a rough estimate of the extent of air contamination.[8] A total of 36 plates were exposed throughout the ship at two-week intervals. Three of the plates had contiguous growth of Penicillium, and one had contiguous growth of yeast. Of the countable colonies there were 72 Penicillium, 11 Aspergillus, 51 yeast, and 2 Rhizopus (black bread mold).

Table I and Figure 2 summarize the weekly breakdown of colony counts. By the

second week submerged (week 6 of patrol cycle) the total number of colonies had decreased considerably. The relative numbers of Penicillium and yeast had also reversed by this time; the Penicillium being predominant during refit and shortly after submerging, the yeasts predominant thereafter. The only plates with contiguous growth were obtained during the first two sampling periods. The sharp peak at the 10th week of the patrol cycle is probably due to field days and clean-ups being held at this time in preparation for returning to port. Large numbers of spores which had settled out in dust were probably propelled back into the air with the increase in activity.

Table I.-Settling Plate Colonies: Summary by Weeks

Type of Fungus	Week of Patrol Cycle						Totals
	2 (Refit)	4 (Submerged)	6	8	10	12 (Surfaced)	
Penicillium	13 (3 contiguous)	35	1	4	15	4	72 (3 contiguous)
Aspergillus	2	5	0	1	3	0	11
Yeast	3	8 (1 contiguous)	13	8	12	7	51 (1 contiguous)
Rhizopus	1	0	0	0	1	0	2
Totals	19 (3 contiguous)	48 (1 contiguous)	14	13	31	11	136 (4 contiguous)

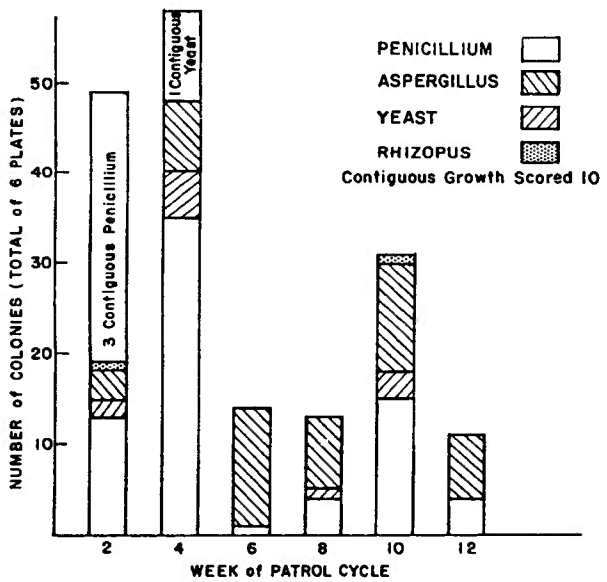

Figure 2. — Settling Plates

Table II and Figure 3 summarize the breakdown of colony counts by area of the ship. Equal numbers of plates were not taken in all areas as it was felt that sampling of some areas was more important. The largest number of fungi were consistently obtained in the mess deck area (9.5 per plate) with yeasts being the predominant organism. The berthing areas and engineering spaces had approximately equal numbers of fungi (approximately 5 per plate) with Penicillium predominating. Lowest levels were found in the missile compartment and scrubber room. Of particular note is the fact that the scrubber room, with the largest number of plates, had the lowest number of fungi cultured (approximately 1.1 per plate). It is felt that no significance can be placed on the high count obtained on the one plate taken in the tunnel as this was taken shortly after submerging when the count was high throughout the ship.

Two plates taken at different periods and placed in the direct air flow from the scrubber and burner outlets grew out only 1 yeast. A plate from the scrubber room exhaust grew 1 Penicillium, and a plate from the scrubber room vent had no growth. Four plates placed in the air flow from ventilators in other areas of the ship had no growth. One plate taken on deck during refit as a control had a contiguous growth of Penicillium, 2 yeast, and 1 Rhizopus; approximately the same proportions as obtained within the ship.

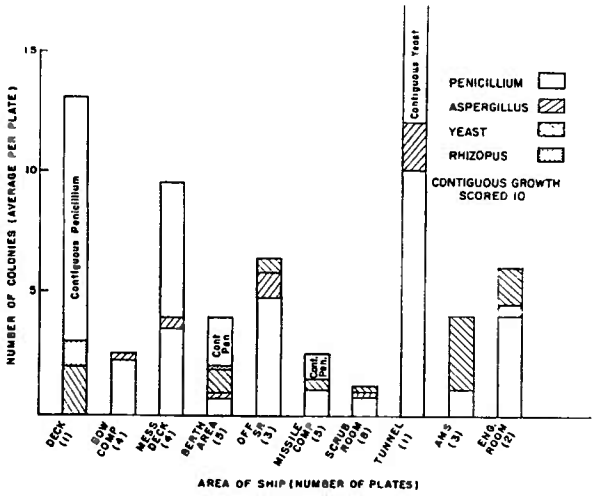

Figure 3. — Settling Plates

Table II.-Settling Plate Colonies: Summary by Area of Ship

Type of Fungus	Area of Ship/Number of Plates: Deck / 1	Bow Comp / 4	Mess Deck / 4	Berth Area / 5	Off S.R. / 3	Missile Comp. / 5	Scrub Room / 8	Tunnel / 1	AMS / 3	Eng. Room / 2	Totals / 36
Penicillium	0 (1 contiguous)	9	14	3 (1 contiguous)	14	5 (1 contiguous	6	10	3	8	72 (3 contiguous)
Aspergillus	0	1	2	1	3	0	1	2	0	1	11
Yeast	2	0	22	5	2	2	2	4 (1 contiguous)	9	3	51 (1 contiguous)
Rhizopus	1	0	0	1	0	0	0	0	0	0	2
Totals	3 (1 contiguous)	10	38	10 (1 contiguous)	19	7 (1 contiguous)	9	16 (1 contiguous)	12	12	136 (4 contiguous)

Part 2: Analysis of Swabs

Swabs planted on slants in 2-ounce medicine bottles were used to analyse the fungal contamination of surfaces on which fungi were likely to be harbored. A total of 30 slants were taken at two-week intervals from various areas throughout the ship. Most of the slants had contiguous growth of organisms after one week: in twenty, yeast predominated; in six, Penicillium predominated. Some Aspergillus and Rhizopus were also present. In only 4 slants were colonies countable.

Table III and Figure 4 summarize the weekly breakdown of cultures. No specific trends in the surface swabs can be noted. Yeast is noted to be the predominant organism on the surface swabs whereas Penicillium predominated on the air plates.

Table IV and Figure 5 summarize the swab cultures by area from which they were taken. Equal numbers of slants were not taken in all areas but dividing the total growth by the number of plates in each area gives a rough estimate of the relative degree of contamination. Only a light growth of yeast was obtained from water from Holy Loch. Heavy growths of yeast were obtained from the bilges, the only other organism being two colonies of Aspergillus. Swabs from the galley and scrubber room both grew large amounts of Penicillium and yeast. In addition, the only Rhizopus on any of the slants were isolated from grates on the exhaust intake and burner intake in the scrubber room. Grates over intakes and exhausts yielded, in general, almost exclusively Penicillium and Rhizopus. Large amounts of yeast were grown from swabs of sink and shower drains and areas around heads.

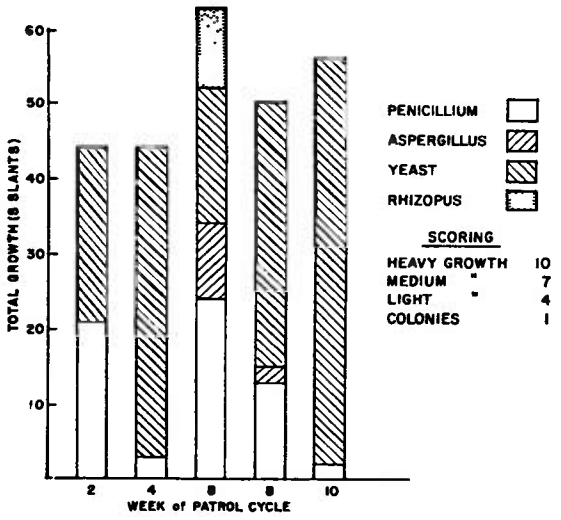

Figure 4. — Slants

Table III -Slants. Summary by Weeks

Type of Fungus	Growth	Week of Patrol Cycle					Totals
		2 (Refit)	4 (Sub-merged)	6	8	10	
Penicillium	Hvy	2	0	2	1	0	5
	Mod.*	0	0	0	0	0	0
	Lt.	0	0	1	0	0	1
	Col.	1	3	0	3	2	9
Aspergillus	Hvy.	0	0	1	0	0	1
	Mod.	0	0	0	0	0	0
	Lt.	0	0	0	0	0	0
	Col.	0	0	0	2	0	8
Yeast	Hvy.	0	3	0	1	3	7
	Mod.	2	1	2	3	2	10
	Lt.	2	1	1	1	0	5
	Col.	1	0	0	0	10	11
Rhizopus	Hvy.	0	0	0	0	0	0
	Mod.	0	0	0	0	0	0
	Lt.	0	0	2	0	0	2
	Col.	0	0	3	0	0	3
Totals	Hvy.	2	3	3	2	3	13
	Mod.	2	1	2	3	2	10
	Lt.	2	1	4	1	0	8
	Col.	2	3	3	5	12	25

Table IV.-Slants: Summary by Source of Culture

Type of Fungus	Growth	Source of Culture/Number of Slants						Totals
		Holy Loch /1	Bilges /3	Galley /6	Scrub Room /6	Sinks Heads /9	Charcoal /5	/30
Penicillium	Hvy.	0	0	2	2	1	0	5
	Mod.	0	0	0	0	0	0	0
	Lt.	0	0	0	0	0	1 *	1
	Col.	0	0	3	4	2	0	9
Aspergillus	Hvy.	0	0	0	0	0	1	1
	Mod.	0	0	0	0	0	0	0
	Lt.	0	0	0	0	0	0	0
	Col.	0	2	0	0	0	0	2
Yeast	Hvy	0	2	1	0	4	0	7
	Mod.	0	1	2	0	3	4	10
	Lt.	1	0	1	2	1	0	5
	Col.	0	0	1	10	0	0	11
Rhizopus	Hvy.	0	0	0	0	0	0	0
	Mod.	0	0	0	0	0	0	0
	Lt.	0	0	0	2	0	0	2
	Col.	0	0	0	3	0	0	3
Totals	Hvy.	0	2	3	2	5	1	13
	Mod.	0	1	2	0	3	4	10
	Lt.	1	0	1	4	1	1	8
	Col.	0	2	4	17	2	0	25

*control -

Five slants were made by spreading charcoal over the surface of the slant The fresh charcoal control yielded only a light growth of Penicillium Cultures from the main (2) and forward (1) filter beds grew only moderate amounts of yeast A sample from the No 1 sanitary tank filter, in addition to a moderate amount of yeast, yielded a heavy growth of Aspergillus A swab from the plates of the galley precipitron, the only one readily accessible to sampling, grew only three colonies of Penicillium

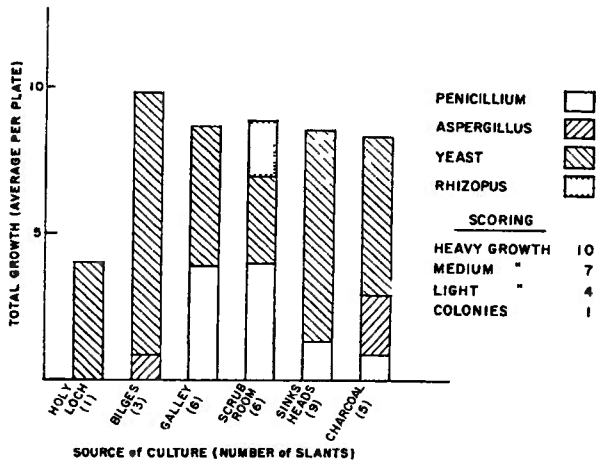

Figure 5 — Slants

DISCUSSION

The data presented provide a good estimation of the character of the fungal ecology of an FBM submarine during prolonged submergence During the refit period the fungal flora do not differ appreciably from that of the local environment, in this case Holy Loch, Scotland After submerging on patrol the total number of air-borne fungi rapidly diminishes to approximately one-third the pre-patrol levels The relative proportions reverse from predominantly Penicillium to predominantly yeast This is probably indicative of the fact that the air is cleansed of spores by the air purification machinery This is further substantiated by the fact that fungi could not be readily cultured from air-flow directly from the scrubbers and burners nor from ventilators throughout the ship The data also indicate that activated charcoal is rather effective in the removal of yeast from the air The relative increase in air-borne yeast is probably due to the large numbers of yeast found to be present in bilges and sinks Growth of yeast is favored by these damp conditions whereas conditions favoring growth of Penicillium or Asperigillus, i e , decaying organic matter, are not readily available

Largest numbers of fungi were consistently cultured from the galley and mess deck area This is as would be expected, for the area not only provides favorable conditions for fungal growth with its ready supply of organic material, but also has a great deal of activity which would tend to stir up spores and propel them into the air Surprisingly, the berthing areas did not have a particularly higher level than other areas of the ship It would have been reasonable to expect that this area might also harbor large numbers of fungi on clothing and shoes These low levels may possibly be due to this area having relatively little traffic to stir up spores

The role of fungi in morbidity among crew members of FBM submarines is probably insignificant Except for dermatomycoses, such as "Athlete's Foot," there is little direct fungal disease among FBM crews Penicillium and Aspergillis are potent allergens but allergic disease is not prevalent among FBM crews Furthermore, by the second week submerged the numbers of these organisms is reduced considerably, therefore reducing the probability of fungal or allergic disease to below the normal local environmental level

Upper respiratory illness is quite common during the in-port period and during the first two weeks of patrol It is quite possible that some of this illness could be on an allergic basis If this be the case, it is quite consistent with the data presented in that the rate of upper respiratory illness decreases in conjuncion with the drop in air-borne fungi It is felt, however, that these are more likely viral illnesses It is quite possible that the atmophere purification machinery is also effective in removing viruses from the air

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13. ABSTRACT

Brief reviews are presented concerning present knowledge of the microscopic flora of the submarine environment, as well as the role of fungal and allergic disease among FBM submarine crews. Data are presented which indicate that the numbers of air-borne fungi are markedly reduced during the course of a submerged patrol, and that this reduction is probably due to the removal of fungi and spores from the air by the air revitalization equipment. It is concluded that fungi probably do not play a significant role in FBM crew morbidity.

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